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Award Number: W81XWH-09-1-0452

TITLE: Preclinical Investigations of a Novel Small Molecule Radiosensitizer of Prostate Cancer

PRINCIPAL INVESTIGATOR: Brian Lally, M.D.

CONTRACTING ORGANIZATION: University of Miami
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REPORT DATE: July 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2011	Annual	1 July 10 – 30 June 2011

4. TITLE AND SUBTITLE

Preclinical Investigations of a Novel Small Molecule Radiosensitizer of Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0452

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Miami
Miami, FL 33136

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The radiation therapy (RT) has proven to be effective at increasing survival of men with prostate cancer. However, the results are far from optimal, with 30-40% of men with intermediate to high risk prostate cancer failing within 5 years. We have been investigating agents that have the potential to enhance the cell killing effects of one or both of these treatments. NS-123 is a drug that we have identified as having such potential.

The objective of any combination of therapeutic agents is to achieve an improved therapeutic gain. The therapeutic gain is a function of both the tumor and normal tissue response. There is no universally accepted measure of a therapeutic result: lifespan, duration of remission, quality of life are all important and reflect different facets of the total result. When therapies are compared, it is necessary to show that one treatment controls the disease better than another for a similar level of toxicity. We recently reported the results of preclinical studies on a novel radiosensitizer, 4'-bromo-3'-nitropropiophenone (NS-123) that we identified using a cell-based, high-throughput screening method. In these studies, NS-123 radiosensitized human lung adenocarcinoma, colon adenocarcinoma, and glioma cells. Recently, we have demonstrated that **NS-123 also radiosensitizes prostate cancer cells**. Importantly, NS-123 appears to be a 'true' radiosensitizer as no overt toxicity was seen in any of the normal tissue models that we studied. Investigations into the mechanisms responsible for this radiosensitization suggest thatNS-123 inhibits the DNA repair pathways, possible as a result of some upstream inhibition within the phosphatidylinositol-3-kinase/Akt pathway. NS-123 appears to sensitize prostate cancer cells with only a short exposure of 1 hr. Animal studies withdaily treatment (50 mg/kg) showed no toxicity. Studies investigating in vivo radiosensitization have been initiated.

15. SUBJECT TERMS
NS-123 radiosensitizes prostate cancer cells

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

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- **INTRODUCTION:**

We reported the results of preclinical studies on a novel radiosensitizer, 4'-bromo-3'-nitropropiophenone (NS-123) that we identified using a cell-based, high-throughput screening method.[1] In these studies, NS-123 radiosensitized human lung adenocarcinoma, colon adenocarcinoma, and glioma cells. As part of this project, we have also found that **NS-123 radiosensitizes prostate cancer cells**. Importantly, NS-123 appears to be a „true" radiosensitizer as no overt toxicity was seen in any of the normal tissue models that we studied. Preclinical investigation of NS-123 has formed the basis for this research proposal. In the last 12 months, Dr. Lally has completed assembly of his research team and they are performing the proposed work. This update represents work that his team has collectively put together.

- **BODY:**

The proposed experiments are to determine the efficacy of NS-123 as a radiosensitizing agent in the treatment of prostate cancer as well as providing a better understanding of the molecular mechanisms responsible for the control prostate cancer. The primary focus of the last 12 months have been to perform the necessary pre-clinical studies *in vivo* and *in vitro*. The ability to exploit this relationship is likely to have significant impact for the care of patients with prostate cancer. Assuming these results are as encouraging as the results previously published, we anticipate developing a clinical trial using this therapeutic rationale.

Specific Aim 1: Maximize the therapeutic gain obtained by combining NS-123 with RT±AD in prostate cancer cells *in vitro*.

RT is one of the most common treatments for clinically localized prostate cancer and is often paired with AD in patients considered to have intermediate to high risk disease. Our recent results[2] support the hypothesis that one of the main reasons for failure in this populations is incomplete eradication of the local disease. Although higher RT doses result in better local outcomes, local control remains an underappreciated problem. If NS-123 improves the therapeutic gain, it could have a significant impact on local control.

The main goal of this Aim is to determine the optimal *in vitro* dose enhancement ratio (DER) that can be obtained by combining NS-123 with RT in prostatate cancer cells. To complete this endpoint, clonogenics were performed with multiple time points.

In **Table 1** at right, we present the results of these clonogenic assays using PC3 cancer cells. As is evident we consistently see radiosensitization as DER at 0.01 of greater than 1.0. Each combination of pre-irradaition (Pre-IR) and post-irradiation (Post-IR) represents the averaged results of at least 2 experiments. Some aspects of the radiosensitization still need to be investigated; however it is reassuring to see that even with minimal pretreatment time (i.e., only 1 hr) of NS-123 radiosensitization is present. The significance of these results relevant to our proposed animal studies is that we will administer NS-123 one hour prior to the planned irradiation.

Table 1. Results of Clonogenic Assays

Pre-IR time (hr)	Post-IR time (hr)	NS-123 (μM)	DER at 0.01
1.0	4.0	20	1.2900
1.0	4.0	30	1.1940
1.0	5.0	20	1.3810
1.0	5.0	30	1.3810
4.0	0.0	10	1.0730
4.0	0.0	20	0.8880
4.0	0.0	20	1.1226
4.0	0.0	30	1.2360
4.0	0.0	20	1.0090
4.0	0.0	30	0.9850
4.0	4.0	20	1.0497
4.0	4.0	30	1.0662
16.0	4.0	20	1.0142
16.0	4.0	30	0.9569
16.5	0.0	20	1.2204
16.5	0.0	30	1.0324
16.5	4.0	20	1.0322
16.5	4.0	30	1.0000

Our *in vitro* studies allowed us to clarify another important issue. Some of the clonogenic assays performed with the varying time we failed to identify radiosensitization, especially when we were using 30 μM NS-123. We reviewed these experiments identified the lack of radiosentisation was function of decreased survival at 0 Gy. NS-123 was functioning more as a cytotoxic agent than as a radiosensitizer. As a result, we investigated lower concentrations of NS-123. Representative plates are presented in Figure 1, on the next page.

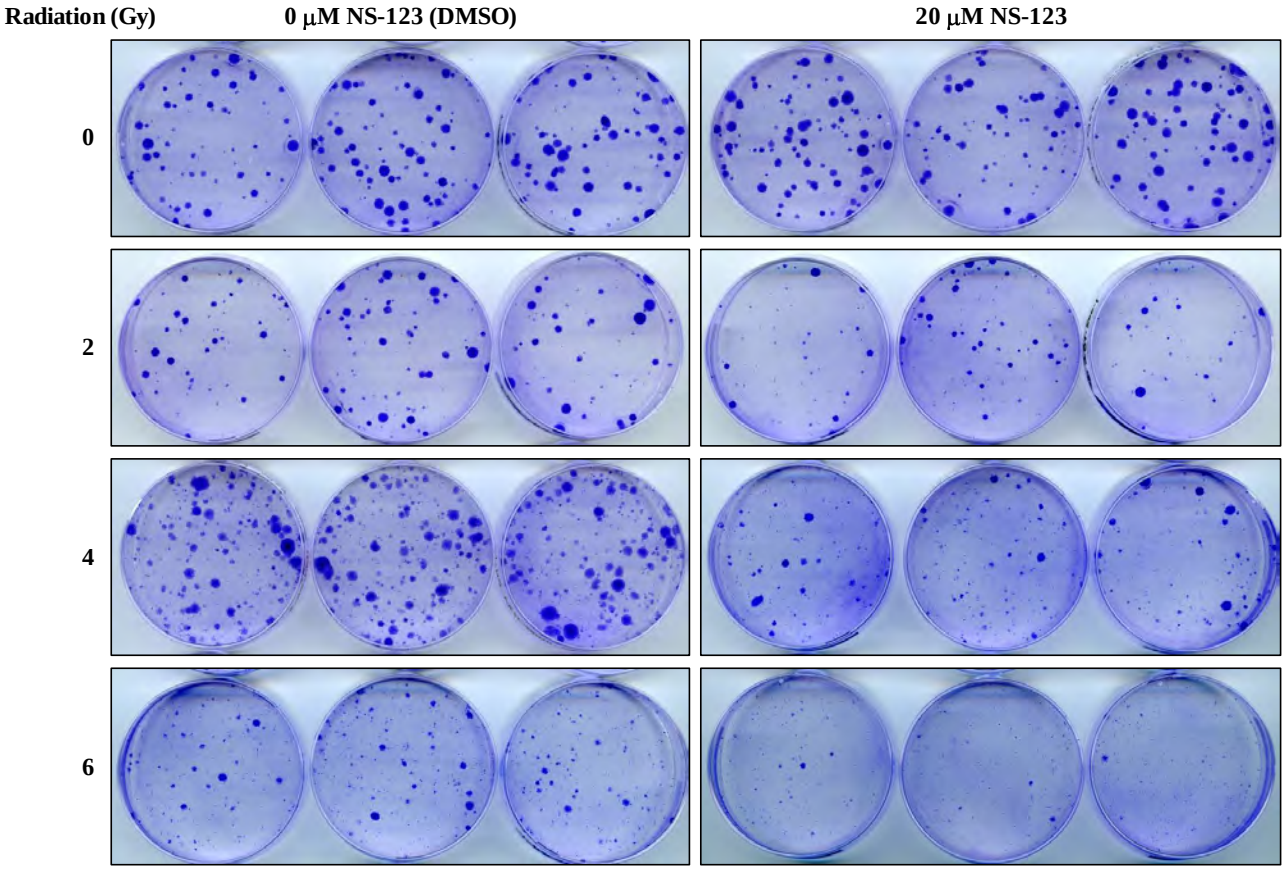

Figure 1. PC3 cells treated with NS-123 for 1 hr, irradiated with a 4 hr recovery period. Cells were trypsinized and plated for colony formation. Colonies were stained with crystal violet after 14 days of growth at 37 °C.

Thus, lower dose of NS-123 are effective at radiosensitizing prostate cancer cells. In the table below, we present the raw data in terms of number of colonies counted. At 0 Gy, no cytoxicity related to NS-123 was identified.

Table 2. PC3 cells treated with NS-123 for 1 hr, irradiated with a 4 hr recovery period. Cells were trypsinized and plated for colony formation. Colonies were stained with crystal violet after 14 days of growth at 37 °C.

	0 μM NS-123 (DMSO)		**20 μM NS-123**	
Radiation (Gy)	**# colonies (avg. 3 plates)**	**# cells plated**	**# colonies (avg. 3 plates)**	**# cells plated**
0	82.0	200	85.3	200
2	58.3	500	33.0	500
4	135.0	6,000	42.7	6,000
6	39.3	10,000	8.0	25,000

Specific Aim 2: Determine if NS-123 can be integrated into current prostate cancer treatment paradigms to produce an increase in the therapeutic gain *in vivo*. We have completed the toxicology study. Five male adult nude (nu/nu) mice were injected with 50 mg/kg of NS-123 daily for 1 week. All mice survived and no weight loss or toxicity was observed. Necropsy was performed; Appedix A shows that no abnormalities were found.

At this point we have initiated *in vivo* studies to study the radiosensitization of NS-123 in male adult nude (nu/nu) mice with implanted tumor cells. Based on results of **Aim 1** and **Aim 2**, 50 mg/kg of NS-123 will be administered 1 hour prior to irradiation of the tumors.

- **KEY RESEARCH ACCOMPLISHMENTS:**
  - NS-123 radiosensitzes prostate cancer cells with a variety of treatment schedules. Radiosentiziation was identified with lower doses of NS-123 and with administration only 1 hr prior to irradiation.
  - NS-123 is well tolerated when given daily for 5 days. No toxicity was identified.

- **REPORTABLE OUTCOMES:**
  - An abstract entitled "Preclinical testing of a novel small molecule radiosensitizer of prostate cancer cells" was presented at the 2011 IMPaCT Innovative Minds in Prostate Cancer Today Conference, sponsored by the Department of Defense Prostate Cancer Research Program. The meeting was held in Orlando, Fl March 9-12, 2011.

- **CONCLUSION:**

Dr. Lally's laboratory has been established and his research team is now in place. A manuscript on NS-123 was submitted for publication. Unfortunately, the manuscript was rejected but is under revision for submission in another journal. The work completed thus far has been very important and has helped define the animal experiments. These studies are now underway to determine the *in vivo* radiosensitization potential of NS-123. With positive results, we will seek to develop a clinical trial for testing in humans.

- **REFERENCES:** List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine*, etc.).

1. Lally, B.E., et al., *Identification and biological evaluation of a novel and potent small molecule radiation sensitizer via an unbiased screen of a chemical library.* Cancer Res., 2007. **67**(18): p. 8791-8799.
2. Morgan, P.B., et al., *Radiation dose and late failures in prostate cancer.* Int J Radiat Oncol Biol Phys, 2007. **67**(4): p. 1074-81.

- **APPENDICES:**
  1. IACUC Approval
  2. UM Comparative Pathology Laboratory Accession

MILLER
SCHOOL OF MEDICINE

Annual Renewal Approval

02-Jun-2011

Dear Dr. LALLY,

The following annual renewal was reviewed and approved by the University of Miami Institutional Animal Care and Use Committee (IACUC) as of the date below:

Protocol Number:	09-112 Renewal 03
Protocol Title:	Preclinical investigations of a novel small molecule radiosensitizer of prostate cancer.
Protocol Sponsor:	DEPT OF DEFENSE
Protocol PI:	LALLY, BRIAN E
Institution:	University of Miami
Date of Approval:	02-Jun-2011
Approval Period:	10-Jun-2011 to 09-Jun-2012

The University of Miami has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), National Institutes of Health. The assurance number is #A-3224-01, effective July 11, 2007. Additionally, as of July 20, 2010, the Council on Accreditation of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International) has continued the University of Miami's full accreditation.

Sincerely,

Sari Izenwasser, PhD

Chair, **Institutional Animal Care and Use (IACUC)**
Professor of Psychiatry and Behavioral Sciences
University of Miami Miller School of Medicine
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Accession # 11-7082 **page 1** **Accession Date: 06/06/2011**

Investigator: Rad Oncology/ Lally
Animal ID: A1, A2, A3 ,A4, & A5

Date to Compath lab: 06/206/2011

Comments:

Enclosed is the histological evaluation of tissues from the 5 mice submitted.
All were Within Normal Limits

A1-1	Brain	Within Normal Limits
A1-2	Sternum & Femur	Within Normal Limits
A1-3	Lung, Heart, Liver w/ Gall Bladder	Within Normal Limits
A1-4	Stomach, Kidney w/ Adrenal, Bladder, Skeletal Muscle	Within Normal Limits
A1-5	Intestinal Roll	Within Normal Limits
A2-1	Brain	Within Normal Limits
A2-2	Sternum & Femur	Within Normal Limits
A2-3	Lung, Heart, Liver w/ Gall Bladder	Within Normal Limits
A2-4	Stomach, Kidney w/ Adrenal, Bladder, Skeletal Muscle	Within Normal Limits
A2-5	Intestinal Roll	Within Normal Limits
A3-1	Brain	Within Normal Limits
A3-2	Sternum & Femur	Within Normal Limits
A3-3	Lung, Heart, Liver w/ Gall Bladder	Within Normal Limits
A3-4	Stomach, Kidney w/ Adrenal, Bladder, Skeletal Muscle	Within Normal Limits
A3-5	Intestinal Roll	Within Normal Limits
A4-1	Brain	Within Normal Limits
A4-2	Sternum & Femur	Within Normal Limits
A4-3	Lung, Heart, Liver w /Gall Bladder	Within Normal Limits
A4-4	Stomach, Kidney w/ Adrenal, Bladder, Skeletal Muscle	Within Normal Limits
A4-5	Intestinal Roll	Within Normal Limits
A5-1	Brain	Within Normal Limits
A5-2	Sternum & Femur	Within Normal Limits
A5-3	Lung, Heart, Liver w/ Gall Bladder	Within Normal Limits
A5-4	Stomach, Kidney w/ Adrenal, Bladder, Skeletal Muscle	Within Normal Limits
A5-5	Intestinal Roll	Within Normal Limits

Norman H. Altman, VMD, DACVP

6/22/2011
Date